# ANATOMY OF THE FEMALE REPRODUCTIVE SYSTEM OF TABANIDAE

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

# CHAPTER I

## INTRODUCTION

Tabanidae comprise a large family of haematophagus flies with many species that are important pests of humans and livestock. Tabanids cause loses in livestock production through annoyance associated with their feeding as reviewed by Steelman (1976), mechanical transmission of disease agents (Krinsky 1976) and blood loss (Hollander and Wright 1980b).

In order to describe reproductive processes of horseflies there have been studies on seasonal parity (Magnarelli 1976, Morris and Defoliart 1971), follicular development (Magnarelli and Anderson 1977), and nectar feeding in relation to oogenesis (Bosler and Hansens 1974). Only one study on the reproductive processes of the Tabanidae of Oklahoma has been done (Ehrhardt 1981).

Snodgrass (1935) gave a general description of the anatomy of dipteran reproductive systems with reference to Tabanidae. Barr (1974) included the tabanids as flies of medical importance in his review of Diptera reproductive systems. Olsufjev's (1977) study on the female reproductive tract of one species from Russia and Ovazza's and Taufflieb's (1954) morphological study on the spermathecae of some African Tabanidae represent the only detailed anatomical and morphological studies on reproductive systems in this group. There are no anatomical or histological descriptions of the reproductive systems for North American Tabanidae. One objective of this study was to provide such descriptions of the

female reproductive system of Tabanus abactor Philip. This species is the most abundant tabanid pest of cattle in northcentral Oklahoma. A second objective was to examine the cellular changes in ovarian follicles and spermathecae during ovipositional cycles of T. abactor. Finally, comparative studies were completed on the structure of spermathecae of the principal tabanid species of Oklahoma.

# CHAPTER II

# ANATOMICAL AND HISTOLOGICAL DESCRIPTION OF THE FEMALE REPRODUCTIVE SYSTEM OF *TABANUS ABACTOR* PHILIP

## INTRODUCTION

Snodgrass (1935) made generalized references to the structure of reproductive tracts of female dipterans in a comparison of the reproductive tracts of insect orders. Clements (1963) reviewed research on the reproductive physiology of Culicidae, and Barr (1974) reviewed reproduction in biting flies, primarily mosquitoes, although some aspects of reproductive processes in Tabanidae were included. Olsufjev (1977) described the female reproductive tract of one species from Russia and Ovazza and Taufflieb (1954) described the morphological characteristics of the spermathecae of certain African Tabanidae. No study has described anatomically or histologically the female reproductive system of North American Tabanidae. This study is a description of the female reproductive system of *T. abactor*, the most abundant tabanid pest of northcentral Oklahoma (Hollander and Wright 1980 a,b).

## MATERIALS AND METHODS

Specimens of *T. abactor* were collected with a modified Malaise trap (Roberts 1976) baited with $CO_2$ gas released by using a Matheson 8® regulator. Living specimens were refrigerated until used in laboratory

studies. Specimens were positioned in wax filled dishes prior to dissection with either the ventral side or lateral areas of the exoskelton removed from proper exposure of the reproductive organs. After removal of exoskeleton a drop of saline (Hays 1953) was placed on the internal organs to prevent dessication during the time required for completion of drawings. An M-5® stereomicroscope with a camera lucida (Wild-Heerburg Instruments Inc.) was used for making drawings.

Tissues for histological studies were excised from living specimens and fixed in Duboscq and Brasil's fluid (Galigher and Kozloff 1971). They were then processed through a standard dehydration series of ethanol solutions, cleared in benzene solutions, infiltrated and embedded in Paraplast® (Fisher Scientific Co., M.P. 56-57$^{o}$C). Spermathecal tissues were cut at 6μ with a rotary microtome. Due to increased difficulty in maintaining intact sections as yolk deposition occurred, ovarian tissues were cut at 8μ for early stages of follicular development and at 15-25μ for later stages: sections were affixed to slides with albumin affixative and stained with standard alum haematoxylin-"Triosin", following standard staining procedures (Galigher and Kozloff 1971). Sections were examined using a Bausch and Lomb® microscope and photomicrographs were made using an Olympus® microscope system.

Terminology used for the description of the spermathecae is that of Ovazza and Taufflieb (1954). Related mosquito reproductive system terminology used in this study is that of Clements (1963).

## RESULTS AND DISCUSSION

The female reproductive system of T. abactor is very similar to that of Culicidae as described by Clements (1963). The ovaries lie laterally in the abdomen and when not distended by fully developed eggs extend from the fourth to sixth abdominal segments. A sheath like suspensory ligament extends anteriorly from the apex of each ovary to the tergum of the second abdominal segment (Figs. 1 and 2). Each ovary is comprised of 100-200 ovarioles. Tabanus abactor has meroistic polytrophic ovarioles typical of most dipterans (Barr 1974). Each ovariole, at the Ib stage of follicular development, is comprised of a germarium, secondary follicle, and primary follicle with the entire ovariole surrounded by two sheaths; a tunica propria and an ovariole sheath (Fig. 3). Lateral oviducts extend posteriad to join the common oviduct in the seventh abdominal segment (Fig. 1). The common oviduct extends ventral to the genital chamber which it joins just anterior to the gonopore (Fig. 2).

The genital chamber is located in the seventh and eighth abdominal segments. This chamber is made up of ovate epithelial cells and surrounded by a thin muscularis (Fig. 4). Arising from the genital chamber are paired accessory glands and three spermathecae. Proximal regions of the accessory glands are enlarged and bulbous in structure. The glands constrict as they loop ventrally and extend between the lateral oviducts. They turn back dorsally and proceed anteriorly over the ovaries to the third abdominal segment. In this segment glands turn posteriad and terminate in the fourth segment (Fig. 2). The accessory gland epithelium is comprised of columnar cells with large basophilic

Figure 1. Ventral View: Female Reproductive System of Tabanus abactor; Odc, Common Oviduct; Odl, Lateral Oviduct; Ov, Ovary; SLg, Suspensory Ligament; Spt, Spermathecae.

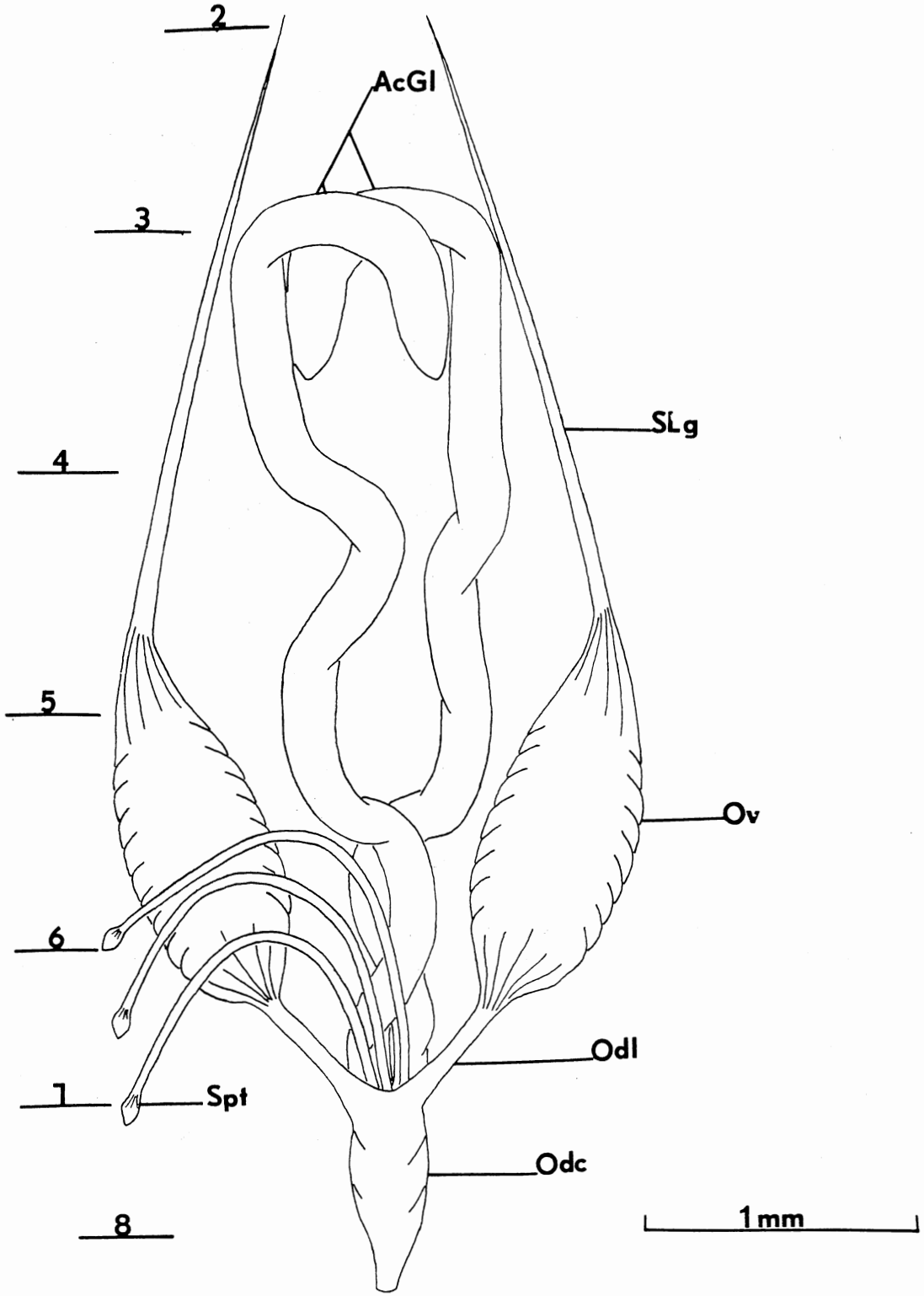
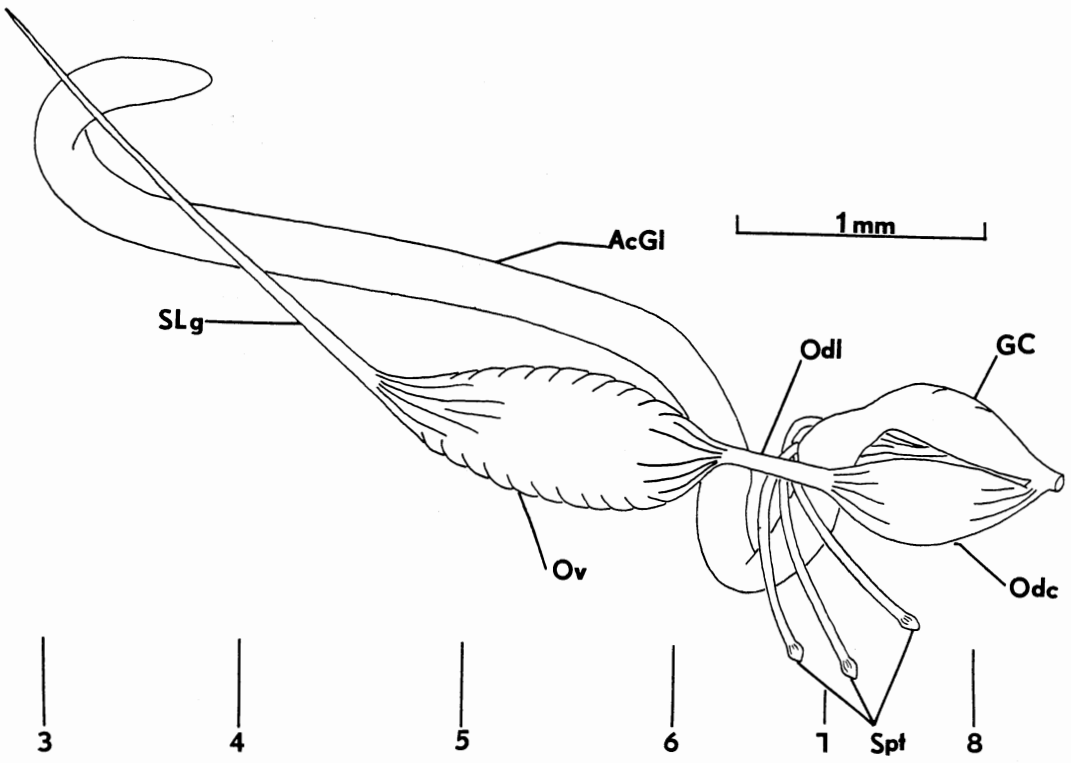

2
AcGl
3
SLg
4
5
Ov
6
Odl
7
Spt
Odc
8
1mm

Figure 2. Lateral View: Female Reproductive System of Tabanus abactor; AcGl, Accessory Gland; GC, Genital Chamber; Odc, Common Oviduct; Odl, Lateral Oviduct; Ov, ovary; Slg, Suspensory Ligament; Spt, Spermathecae.


1mm
AcGl
SLg
Odl
GC
Ov
Odc
3
4
5
6
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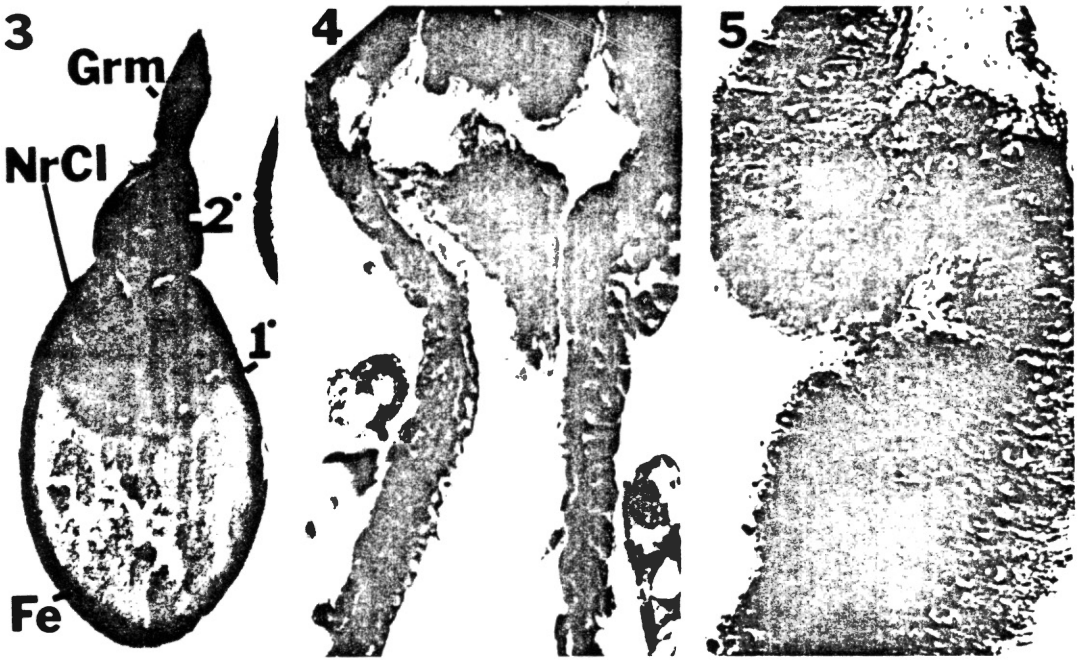
Spt
8

Figure 3. Longitudinal Section of an Ovariole of T. abactor; Grm, Germarium; $1^{o}$, Primary Follicle; $2^{o}$, Secondary Follicle; Fe, Follicular Epithelium; NrCl, Nurse Cells; Y, Yolk. (146X)

Figure 4. Longitudinal Section of the Genital Chamber of Female T. abactor. (489X)

Figure 5. Longitudinal Section of the Accessory Gland of Female T. abactor. (611X)


3
Grm
NrCl
2
1
Fe
4
?
5

nuclei (Fig. 5).

The spermathecae arise from a common duct which enters the genital chamber ventrally between the posterior arms of the genital fork (Figs. 2 and 6). This duct subdivides to form the three spermathecae near the genital chamber. Each spermathecal diverticulum consists of a heavily chitinized basal region or sleeve, a slender duct, and a terminal capsule. Spermathecal ducts extend anteriorly between the lateral oviducts and accessory glands and then fold posteriorly at the seventh abdominal segment with the terminal capsules resting adjacent to the body wall ventral to the common oviduct (Fig. 2).

Spermathecae are comprised of cellular sheaths lined internally by chitinous intima and, exteriorly by a thin muscularis. The basal sleeve region is heavily sclerotized with a distal cupule (Fig. 7). Proximal to the cupule, the chitinous lining of the sleeve is surrounded by lanceolate epithelial cells and an exterior muscularis (Fig. 7). Epithelial cell of this duct region are cuboidal in shape and the chitinous intima is relatively thin (Fig. 9). The terminal capsule is heavily sclerotized with the surrounding sheath comprised of large columnar epithelial cells and muscularis (Fig. 9).

The female reproductive tract of Tabanidae is similar to that of Culicidae and a Calliphoridae. The following comparisons are made from descriptions of Culicidae from Clement's (1963) review on mosquito physiology and Hall's (1948) review of North American blowflies. The ovaries of the three groups of flies are oval in shape and located in the posterior end of the abdominal cavity. The number of ovarioles found in T. abactor is more similar to Culicidae than to Calliphoridae. The lateral oviducts in all three groups of flies unite with the common

Figure 6. Ventral View: Female Reproductive System of Tabanus abactor Genital Chamber Region. AcGl, Accessory Gland; GC, Genital Chamber; GF, Genital Fork; SpC, Spermathecal Cupule; SpTc, Spermathecal Terminal Capsule; Spsh, Spermathecal Sheath; SpD, Spermathecal duct.

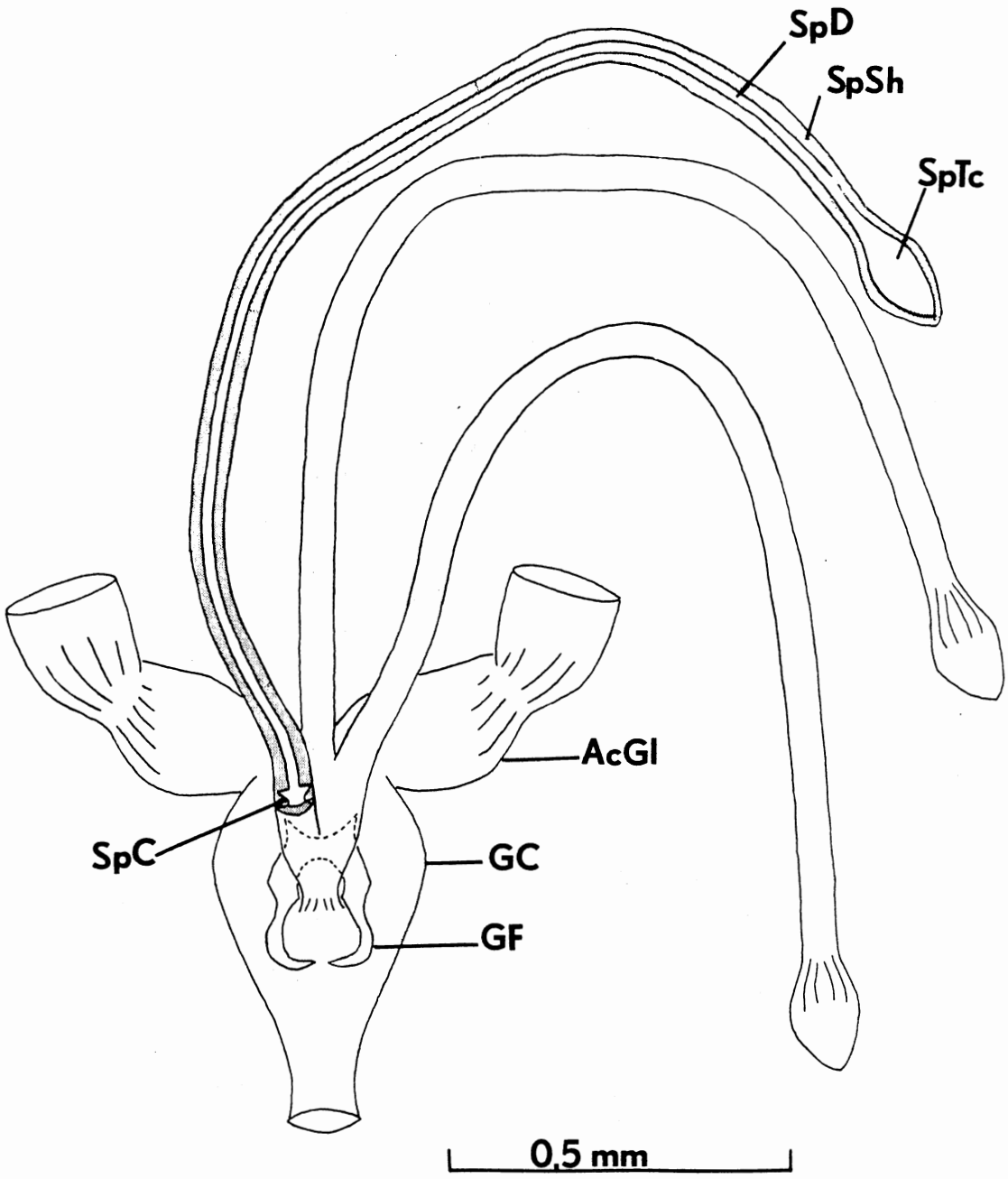
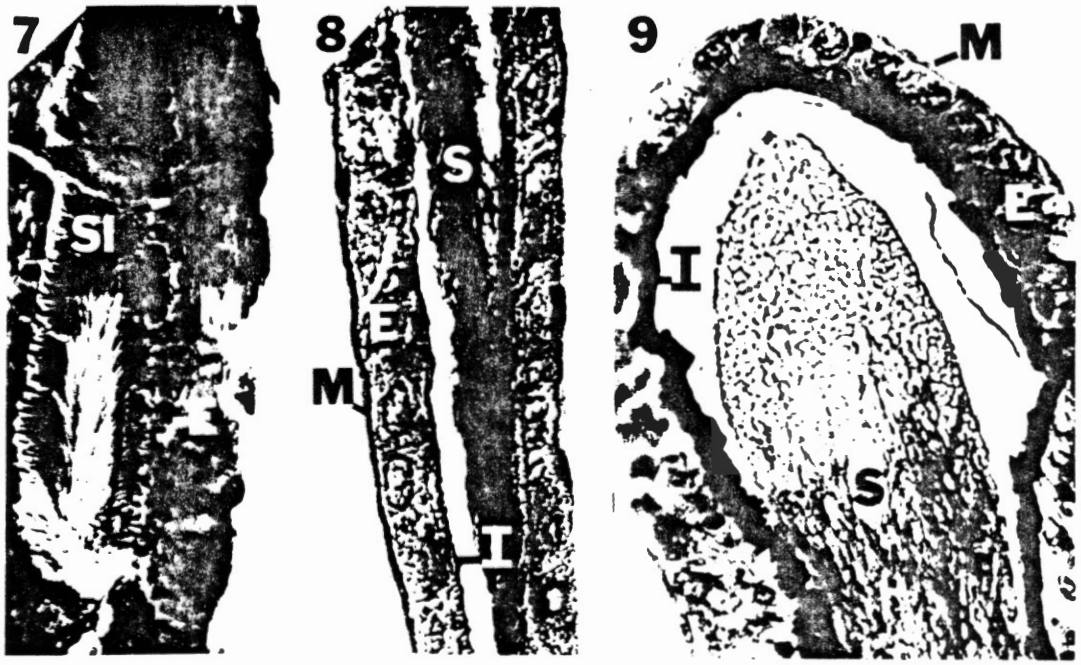

SpD
SpSh
SpTc
AcGl
SpC
GC
GF
0,5 mm

Figure 7. Longitudinal Section of the Sleeve Region of a Spermatheca of Tabanus abactor; M, muscularis; E, epithelium; Sl, sleeve. (381X).

Figure 8. Longitudinal Section of the Duct of a Spermatheca of Tabanus abactor; M, muscularis; E, epithelium; I, intima; S, spermatozoa. (526X).

Figure 9. Longitudinal Section of the Terminal Capsule of a Spermatheca of Tabanus abactor; M, muscularis; E, epithelium; I, intima; S, spermotoza. (500X).


7
SI
8
S
E
M
I
9
M
I
S

oviduct ventral to the genital chamber.

The genital chamber varies considerably in size and cellular composition in these three groups of dipterans. The chamber in T. abactor is much longer than that of the Culicidae. The chamber region of T. abactor is not sclerotized while that of the Calliphoridae is sclerotized in the posterior one third and referred to as an uterovagina (Hall 1948).

Three spermathecae enter the genital chamber ventrally in T. abactor as occurs in all Calliphoridae and the genera Culex, Culiseta, and certain Aedes of Culicidae. However, the spermathecae of T. abactor unite to form a common spermathecal duct before entering the genital chamber which is not the case in Calliphoridae or Culicidae.

The elongate accessory glands of T. abactor are similar in structure to those in Calliphoridae while these organs in Culicidae are short, oval glands. Tabanus abactor has no chitinous lining in these glands like that found in Culicidae or Calliphoridae. There is no bursa copulatrix in either T. abactor or Calliphoridae as found in Cullicidae. A comparison of the three female reproductive tracts of these Diptera shows more similarily between that of Tabanus abactor and the Calliphoridae than between the Culicidae and T. abactor.

# CHAPTER III

# CELLULAR CHANGES IN THE OVARIAN FOLLICLES AND SPERMATHECAE IN TABANUS ABACTOR PHILIP DURING OVIPOSITIONAL CYCLE

## INTRODUCTION

Many studies involving several species of Tabanidae have been done to examine seasonal parity rates (Lane and Anderson 1981), (Magnarelli 1976), (Morrison and DeFoliart 1971), (Thompson et al. 1979) and (Troubridge and Davies 1975). There have been studies done on the autogenous state of some tabanids (Magnarelli and Anderson 1977) and (Thomas 1969, 1972) along with studies examining ovarian development of select species (Lake and Burger 1980) and (Magnarelli and Pechuman 1975). There has been only one each study involving the Tabanidae of Oklahoma (Ehrhardt 1981).

The first objective of this study was to describe follicular development of Tabanus abactor during an ovipositional cycle. This objective was accomplished through a determination of the yolk desposition rate in primary follicles, changes in the follicular epithelial cell shape and a detailed histological examination of relic formation. A second objective was to describe the spermatozoal concentration and movement in the spermathecae through the development cycle of the primary follicles, from the earliest distinguishable stage to post-oviposition. The final objective was to examine the resting stage from uniparous, biparous,

and triparous specimens to find if any changes in the ovarioles occurred with multiple oviposition.

## MATERIALS AND METHODS

Tabanus abactor specimens were collected on June 16 and August 1, 1981 from a Jersey cow. One group were allowed to feed while others were captured before feeding. Both fed and unfed specimens were removed from the cow by covering them with a clear inverted plastic cup as described by Hollander and Wright (1980b). Specimens were placed in a cooler, and transported to the laboratory where they were placed in one liter cartons with cloth mesh tops at ten flies per carton. They were fed a 10% solution of fructose, sucrose and glucose which was similar in composition to nectar found in nature (Wykes 1952) and (Percival 1961). The flies were maintained at 16 hour photophase, $26^{o} \pm 1^{o}C$ and 55-60% RH.

Ten fed flies were dissected on each of ten consecutive days to examine all ovarian stages. Ten non-blood fed specimens were dissected on the initial day of capture and at eight and ten days after capture. For histological studies the reproductive system was removed intact from living specimens and the ovaries and spermathecae were excised and fixed in Dubosq and Brasil's fluid (Galigher and Kozloff 1971). The tissues were dehydrated through a series of ethanol solutions, cleared in benzene and infiltrated and embedded in Paraplast® (Fisher Scientific Co., mp $56-57^{o}C$). Spermathecal tissues were cut at 6µ with a rotary microtome. Ovarian tissues were cut at 8µ during early stages of follicular development and at 15-25µ during later stages. Sections were affixed to slides with albumin affixative. Sections were stained with standard alum haemotoxylin-"Triosin" following standard staining

procedures (Galigher and Kozloff 1971). All sections were examined using a Bausch and Lomb® microscope and photomicrographs were made using an Olympus® microscope system.

## RESULTS AND DISCUSSION

Stages of follicular development were observed and classified using the terminology of Christopher (1911), with modification by Mer (1936) and Detinova (1962). There are four stages of follicular development preceding oviposition and subsequent relic formation after eggs are laid in I. abactor. The general classification scheme involves: stage I, no yolk granules in the oocyte; stage II, yolk granules present, but the nucleus not obscured; stage III, yolk obscures the nucleus, but follicles remains oval in shape; stage IV, the follicle elongated and nearly the shape of the egg when laid. The stage descriptions following, are based on the examination of histological slides of ovarioles of I. abactor. There is a definite pattern in yolk deposition, follicular epithelial cell changes and nurse cell-ovum size ratio during the ovipositional cycle.

Stage Ia: One specimen of an unmated fly exhibited this earliest ovarian stage of development, which is found in the primary follicles of newly emerged specimens only. There is no differentiation of the nurse cells or ova and the follicular epithelium has not formed (Fig. 10). As this specimen was unmated, spermathecae are void of spermatoza (Fig. 11).

Stage Ib: Within ovarioles of mated specimens, nurse cells and the ova can be differentiated in the primary follicles and

Figure 10. Longitudinal Section of the Ovariole at Ia Stage of Follicular Development of Tabanus abactor. (300x).

Figure 11. Longitudinal Section of the Spermathecae of Female Tabanus abactor at Ia Stage of Development. (164x).

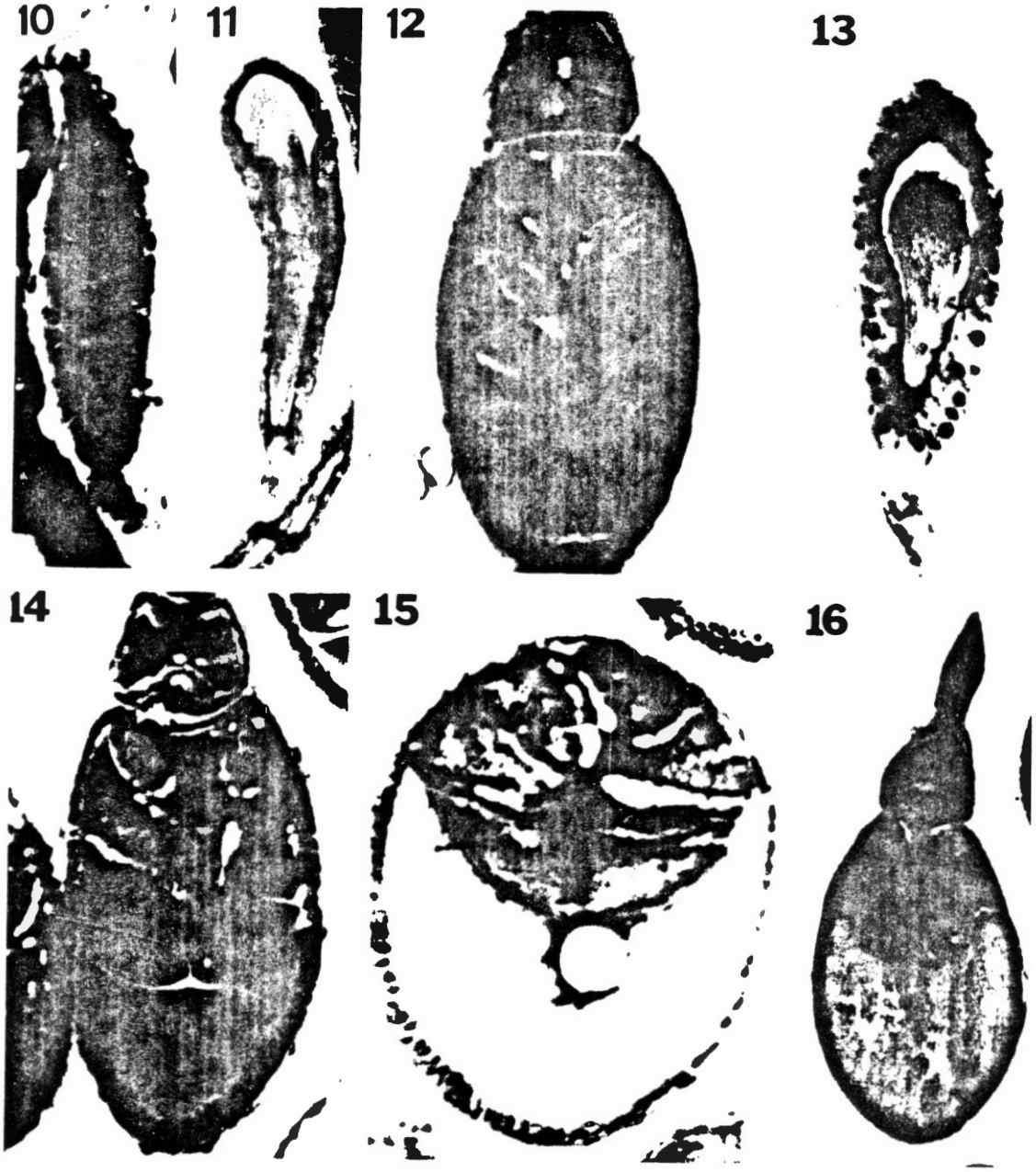
Figure 12. Longitudinal Section of the Ovariole at Ib Stage of Follicular Development of Tabanus abactor. (275x).

Figure 13. Longitudinal Section of the Spermathecae of Female Tabanus abactor at Ib Stage of Development. (289x).

Figure 14. Longitudinal Section of the Ovariole at IIa Stage of Follicular Development of Tabanus abactor. (292x).

Figure 15. Longitudinal Section of the Ovariole at IIb Stage of Follicular Development of Tabanus abactor. (271x).

Figure 16. Longitudinal Section of the Ovariole at IIIa Stage of Follicular Development of Tabanus abactor. (146x).


10
11
12
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14
15
16

follicular epithelial cells nearest the pedicel are columnar in shape while those nearest the germarium are cuboidal. No yolk granules are found in the follicle (Fig. 12). The spermathecae contain spermatozoa throughout with the greatest concentration in the terminal capsule (Fig. 13).

Stage IIa: Follicles are approximately 200μ in length with the nurse cells occupying 126 to 148μ of the intrafollicular area. The ovum occupies the remaining intrafollicular area. Yolk granules have begun to appear along the periphery of the ovum near the epithelium. Follicular epithelial cells nearest the pedicel remain columnar in shape while those near the germarium are cuboidal (Fig. 14).

Stage IIb: The ovarioles of T. abactor develop to this stage known as the resting stage and remain in this stage until a blood meal is taken. Such a resting stage is also found in mosquitoes (Clements 1963) and other hematophagous insects (Detinova 1962). The term resting stage is used because the follicles cease development at this stage and do not advance until a blood meal has been taken. The area within the follicle occupied by ova and nurse cells is approximately the same as in stage IIa, with an increased amount of yolk deposition. The follicular epithelial cells nearest the pedicel are columnar and make up three quarters of the follicular epithelium with the remainder of the epithelium nearest the germarium flattened in shape (Fig. 15).

Spermatozoa are found throughout the spermathecae.

Stage IIIa: After a blood meal is taken, follicles develop beyond the resting stage as described by Bellamy and Bracken (1971) for mosquitoes. The primary follicle is approximately 320μ in length with the ovum occupying 173 to 193μ of the intrafollicular area. The nurse cells occupy the remaining space. The follicular epithelial cells nearest the pedicel are columnar and make up the shape of two-thirds of the epithelium with the remainder of the epithelial cells nearest the germarium flattened in shape (Fig. 16).

Stage IIIb: The primary follicles are approximately 400μ in length with the ovum occupying 219 to 229μ of the intrafollicular area. The nurse cells occupy the remaining space. The follicular epithelial cells nearest the pedicel make up two thirds of the epithelium and are cuboidal in shape. The remainder of the epithelium nearest the germarium is made up of cells flattened in shape. Each ovariole contains a secondary follicle in stage Ib of development which has been described.

Stage IVa: The greatly elongated primary follicle has reached approximately 800μ in length with 712 to 752μ of the intrafollicular area occupied by the ovum while the space occupied by the nurse cells has been greatly reduced. The follicular epithelial cells are primarily flattened with only a few epithelial cells nearest the pedicel cuboidal in shape. Spermatozoa are found in the terminal capsule and basal

sleeve region, with none found in the duct region. It is at this stage of development that the majority of the spermatozoa in the spermatheca are apparently concentrated at the sleeve region where they remain until the ova in the primary follicle reach maturity.

Stage IVb: Egg formation is completed at this stage with the follicle approximately 1.0 mm in length and only remnants of nurse cells discernable. The entire follicular epithelium is flattened. The secondary follicle of each ovariole is in stage Ib of development. The spermatozoal concentration is greatest at the sleeve region, lowest at the terminal capsule with none found in the duct region. I believe that the sleeve acts as an injection system of spermatozoa into the genital chamber just prior to passage of eggs through the common oviduct.

Relic Formation ("sac"-stage): Immediately following oviposition the the follicular epithelial cell remnants form a "sac" within the pedicel of the ovariole (Fig. 17). What had been the secondary follicle in each ovariole now progresses as the primary follicle to stage IIa. Spermatozoa are found throughout the spermathecae of specimens in this condition. If it is assumed that T. abactor mates only once, then after oviposition a redistribution of spermatozoa occurs throughout the spermatheca.

Figure 17. Longitudinal Section of the Ovariole at "sac" Stage Following Oviposition of Tabanus abactor. (225x).

Figure 18. Longitudinal Section of the Ovaiole at "relic" Stage Following Oviposition of Tabanus abactor. (500x).

17

18

Follicular relic ("yellow body" stage): The follicular remnant starts starts to coalesce and cell structure becomes less distinct. The succeeding follicle has now developed to stage IIb at which it remains until the fly is successful in obtaining another blood meal. The follicular remnant completely coalesces into a "yellow body" relic 58μ to 72μ in diameter in which few distinct cellular remnants remain (Fig. 18). Follicles within specimens not allowed to take a second blood meal developed to stage IIb, the resting stage, but never beyond. Follicles remained in IIb resting stage and did not regress. Uniparous, biparous and triparous specimens from a mark-recapture study (Wright unpublished) remained at stage IIb when held for 5 days under laboratory conditions in the absence of another blood meal.

In this histological study certain anatomical and physiological changes have been observed over the follicular developmental cycle. With increased development of the ovum the follicular epithelial cells progress from columnar to cuboidal to flattened. This process of cell flattening begins at the end of the follicle toward the germarium and is completed at the pedicel. The spermatozoa distribution within a spermatheca is evenly located until the follicle nearly reaches maturity, then a concentration of spermatozoa occurs in the sleeve region. It is speculated that the sleeve acts as a mechanism to force spermatozoa into the genital chamber as eggs pass through the oviduct.

The resting stage, IIb of the ovariole occurs with each follicular cycle. A blood meal is required for completion of each follicular cycle.

A blood meal is required for completion of each follicular cycle, although condition of follicles does not regress in the absence of a blood meal. The "yellow body" relic is comprised of the cellular remnants of the follicular epithelium which coalesce into a single mass. From the descriptions based on these anatomical and histological characteristics, field captures specimens of T. abactor can be precisely age graded as to their ovarian stage, except when in the sac stage.

# CHAPTER IV

## A MORPHOLOGICAL STUDY OF THE SPERMATHECAE OF TABANIDAE (DIPTERA) IN OKLAHOMA

Oldroyd (1952) recognized that spermathecal shapes varied among tabanid species and could be used as an aid in species identification. Ovazza and Taufflieb (1954) showed that Tabanidae of French Equatorial Africa could be identified to genus and species utilizing only spermathecal characteristics. Little descriptive information exists relating to the spermathecae of North American Tabanidae except that there are three in number (Downes 1968).

The first objective of this study was to describe anatomically the spermathecae of certain Tabanidae. A second objective was to determine if anatomical characteristics of spermathecae could be used in species identification.

### MATERIALS AND METHODS

The spermathecae of 18 species of Oklahoma Tabanidae were examined and described. Specimens were collected with modified Malaise traps (Roberts 1976), baited with $CO_2$ gas released by using a Matheson 8® regulator. A total of 25 specimens of each species collected throughout the season were examined except in ten instances where fewer specimens were collected.

Living specimens were placed in a wax filled dish with the ventral

side exposed for removal of sternites from the three terminal abdominal segments. As the reproductive system was exposed, the spermathecae, which lie adjacent to the ventral body wall, were carefully detached from the genital chamber. Upon removal from the insect, the spermathecae were placed in a drop of saline (Hays 1953) on a slide and covered with a coverslip. They were examined immediately and photomicrographs were made with a phase-contrast microscope. Measurements of certain regions of the spermathecae were made and included total length, sleeve width, terminal capsule length and width. Measurements were made with an ocular micrometer. The areas measured were regions determined to have the greatest variation between species and uniformity within a species, thus improving their utility for identification purposes.

Photographs of the spermathecal terminal capsules were taken at 100x. Photographs of sleeve regions were taken at 200x but photos are probably not this magnification. The first terminal capsule shape mentioned for each species was the dominant shape present.

## RESULTS AND DISCUSSION

Spermathecae of all species have three distinct regions; a basal sleeve, an elongate duct and a terminal capsule (Fig. 19). As terminology relating to shape of the terminal capsule did not previously exist, a system has been developed recognizing four basic shapes; ovate, tubular, lanceolate and diamond (Fig. 20). Table I lists the Tabanus spp. arranged from shortest to longest spermathecal length. Table II lists all other tabanid species examined arranged in a similar manner. Also listed in these tables are means (mm) and standard deviations for all spermathecal measurements for each species. The

Figure 19. Longitudinal Section of Spermatheca of Tabanidae (semi-diagrammatic). Sp C, spermathecal cupule, (sleeve width measurement); SpTc, spermathecal terminal capsule (capsule length measurement); SpSh, epithelial cells; SpSl, spermathecal sleeve; SpD, spermathecal duct.

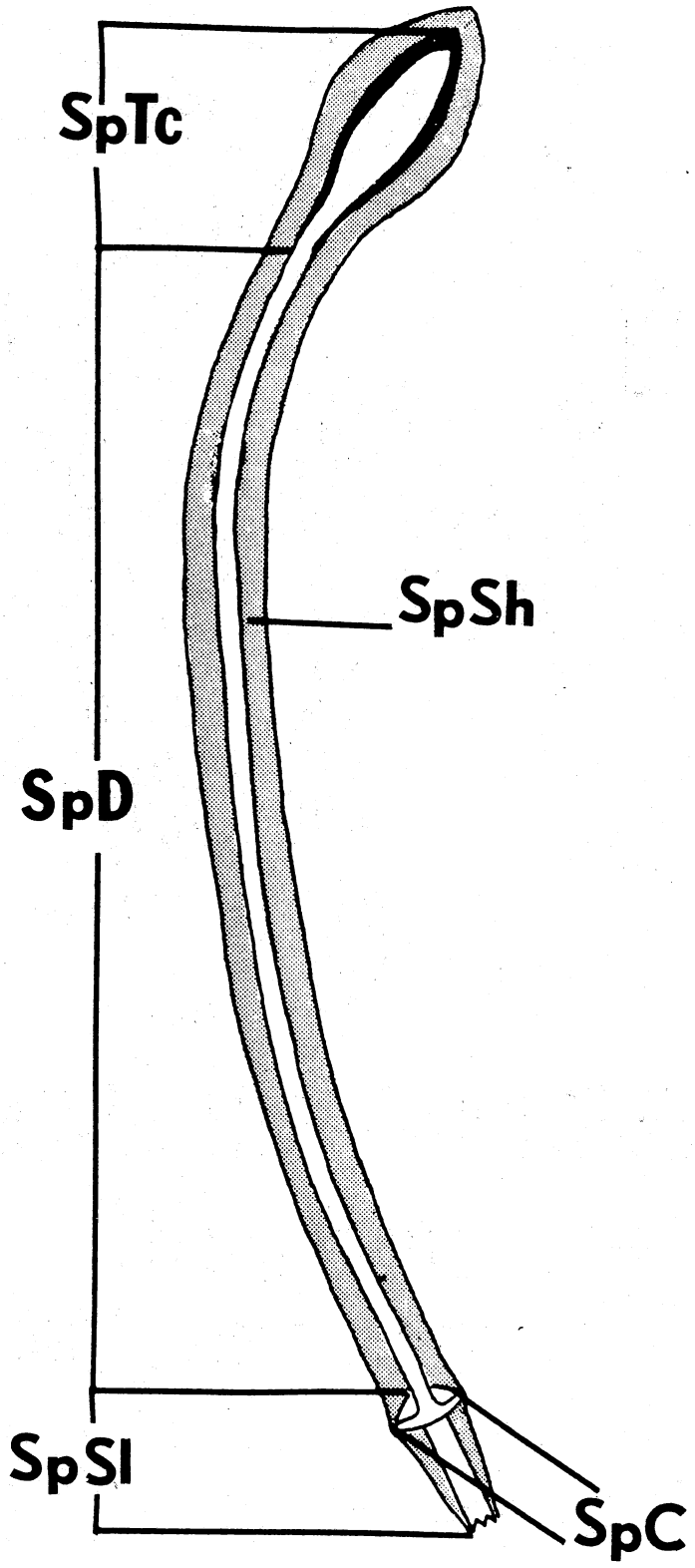
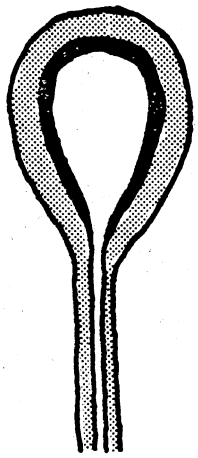
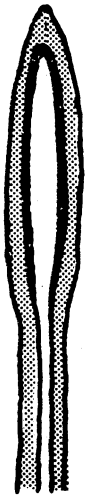

SpTc
SpSh
SpD
SpSl
SpC

Figure 20. Shapes of Terminal Capsules in Spermathecae of Tabanidae (diagrammatic). A, oval; B, diamond; D, tubular; D, lanceolate.



A

B



C

D

TABLE I

MEANS AND STANDARD DEVIATIONS FOR MEASUREMENTS OF SPERMATHECAL REGIONS IN *TABANUS* SPECIES

| Species | Total Dissected | Total length (mm) | Terminal Capsule length(mm) | Terminal Capsule width(mm) | Sleeve Width (mm) |
|---|---|---|---|---|---|
| *T. subsimilis* | 25 | 1.42 ± 0.150 | 0.218 ± 0.027 | 0.119 ± 0.016 | 0.060 ± 0.008 |
| *T. venustus* | 9 | 3.84 ± 0.156 | 0.266 ± 0.028 | 0.135 ± 0.019 | 0.116 ± 0.010 |
| *T. nigripes* | 10 | 4.11 ± 0.211 | 0.335 ± 0.052 | 0.071 ± 0.013 | 0.090 ± 0.007 |
| *T. atratus* | 25 | 4.35 ± 0.347 | 0.360 ± 0.033 | 0.209 ± 0.025 | 0.120 ± 0.011 |
| *T. abactor* | 25 | 4.44 ± 0.314 | 0.217 ± 0.027 | 0.087 ± 0.012 | 0.081 ± 0.012 |
| *T. stygius* | 16 | 5.29 ± 0.258 | 0.303 ± 0.063 | 0.157 ± 0.024 | 0.123 ± 0.021 |
| *T. mularis* | 25 | 5.63 ± 0.235 | 0.258 ± 0.019 | 0.075 ± 0.010 | 0.093 ± 0.006 |
| *T. sulcifrons* | 25 | 6.49 ± 0.252 | 0.030 ± 0.028 | 0.130 ± 0.016 | 0.119 ± 0.008 |
| *T. calens* | 12 | 7.50 ± 0.308 | 0.319 ± 0.036 | 0.114 ± 0.018 | 0.119 ± 0.011 |
| *T. trimaculatus* | 18 | 8.14 ± 0.408 | 0.411 ± 0.019 | 0.243 ± 0.053 | 0.045 ± 0.019 |
| *T. quaesitus* | 25 | 8.75 ± 0.368 | 0.277 ± 0.020 | 0.630 ± 0.008 | 0.106 ± 0.012 |
| *T. equalis* | 25 | 8.99 ± 0.468 | 0.345 ± 0.026 | 0.079 ± 0.010 | 0.135 ± 0.012 |

TABLE II

MEANS AND STANDARD DEVIATIONS FOR MEASUREMENTS OF SPERMATHECAL REGION IN TABANIDAE OTHER THAN *TABANUS* SPECIES

| Species | Total Dissected | Total length (mm) | Terminal Capsule length(mm) | Terminal Capsule width(mm) | Sleeve Width (mm) |
|---|---|---|---|---|---|
| *Chrysops flavidus* | 20 | 1.16 ± 0.066 | 0.204 ± 0.015 | 0.077 ± 0.014 | 0.025 ± 0.003 |
| *C. callidus* | 18 | 1.37 ± 0.109 | 0.217 ± 0.040 | 0.092 ± 0.010 | 0.030 ± 0.003 |
| *Silvius quadrivittatus* | 12 1 | 1.66 ± 0.042 | 0.227 ± 0.023 | 0.110 ± 0.010 | 0.024 ± 0.003 |
| *Esenbeckia incisuralis* | 2 | 3.03 ± 0.218 | 0.847 ± 0.077 | 0.199 ± 0.145 | a/ |
| *Anacimas dodgei* | 15 | 3.26 ± .150 | 0.347 ± 0.032 | 0.113 ± 0.015 | 0.077 ± 0.005 |
| *Hybomitra lasiophthalma* | 25 | 5.44 ± .243 | 0.381 ± 0.055 | 0.089 ± 0.022 | 0.133 ± 0.014 |

a/ *E. incisuralis* has no capule region

following terminology was used in the descriptions of the basal sleeve, duct and terminal capsule of each species spermatheca.

Terms relating to sleeve region:

Cupule: sclerotized distal region of the sleeve.

Bulbous cupule: distal end of cupule has an enlarged circular area.

Diamond cupule: diamond shape formed at the distal end of sleeve where it joins the duct.

Inverted semi-circular cupule: distal end of sleeve is semi-circular in shape, with rounded edge oriented proximally.

Kidney cupule: distal end of cupule bean shaped.

Lateral horns of cupule: proximal end of cupule forms lateral projections.

Oval cupule: distal end of sleeve oval in shape.

Semi-circular cupule: distal end of sleeve is semi-circular in shape, with rounded edge oriented distally.

Tapered point: promixal end of sleeve forms a point.

Three prongs proximally: proximal end of sleeve with a three pointed crown shape.

Two prongs proximally: proximal end of a sleeve forms a two pointed crown shape.

Terms relating to terminal capsule:

Intima with longitudinal impressions: intima of terminal capsule with longitudinal indentations on the outer surface.

Intima smooth: intima of terminal capsule without indentations or ridges on the outer surface.

Intima sculptured: intima of terminal capsule with elongate, transverse indentations on the outer surface.

Intima highly chitinous: intima very dark and thick.

Intima moderately chitinous: intima dark and of medium thickness.

Intima lightly chitinous: intima light and thin.

Spermathecacae of Tabanidae species are described as follows:

Tabanus subsimilis Bellardi

Sleeve: three prongs proximally; semi-circular cupule (Fig. 21a).

Duct: curved; bulbous

Terminal capsule: ovate; smooth; intima highly chitinous (Fig. 21b).

Tabanus venustus Osten Sacken

Sleeve: three prongs proximally; semi-circular cupule (Fig. 21c).

Duct: straight; not bulbous

Terminal capsule: ovate or occasionally diamond; smooth, intima moderately chitinous (Fig. 21d).

Tabanus nigripes Wiedemann

Sleeve: two prongs proximally; semi-circular cupule (Fig. 21e).

Duct: straight; not bulbous

Terminal capsule: tubular or occasionally lanceolate; smooth, intima moderately chitinous (Fig. 21f).

Tabanus atratus F.

Sleeve: three prongs proximally; circular or oval cupule (Fig. 21g).

Duct: curved; not bulbous.

Figure 21. Photomicrographs of the Sleeve (200x) and Terminal Capsule (100x) Regions of Nine Species of Tabanidae.

Figure 21a. Sleeve of Tabanus subsimilis;

Figure 21b. Terminal Capsule of T. subsimilis;

Figure 21c. Sleeve of T. venustus;

Figure 21d. Terminal Capsule of T. venustus;

Figure 21e. Sleeve of T. nigripes;

Figure 21f. Terminal Capsule of T. nigripes;

Figure 21g. Sleeve of T. atratus;

Figure 21h. Terminal Capsule of T. atratus;

Figure 21i. Sleeve of T. abactor;

Figure 21j. Terminal Capsule of T. abactor;

Figure 21k. Sleeve of T. stygius;

Figure 21l. Terminal Capsule of T. stygius;

Figure 21m. Sleeve of T. mularis;

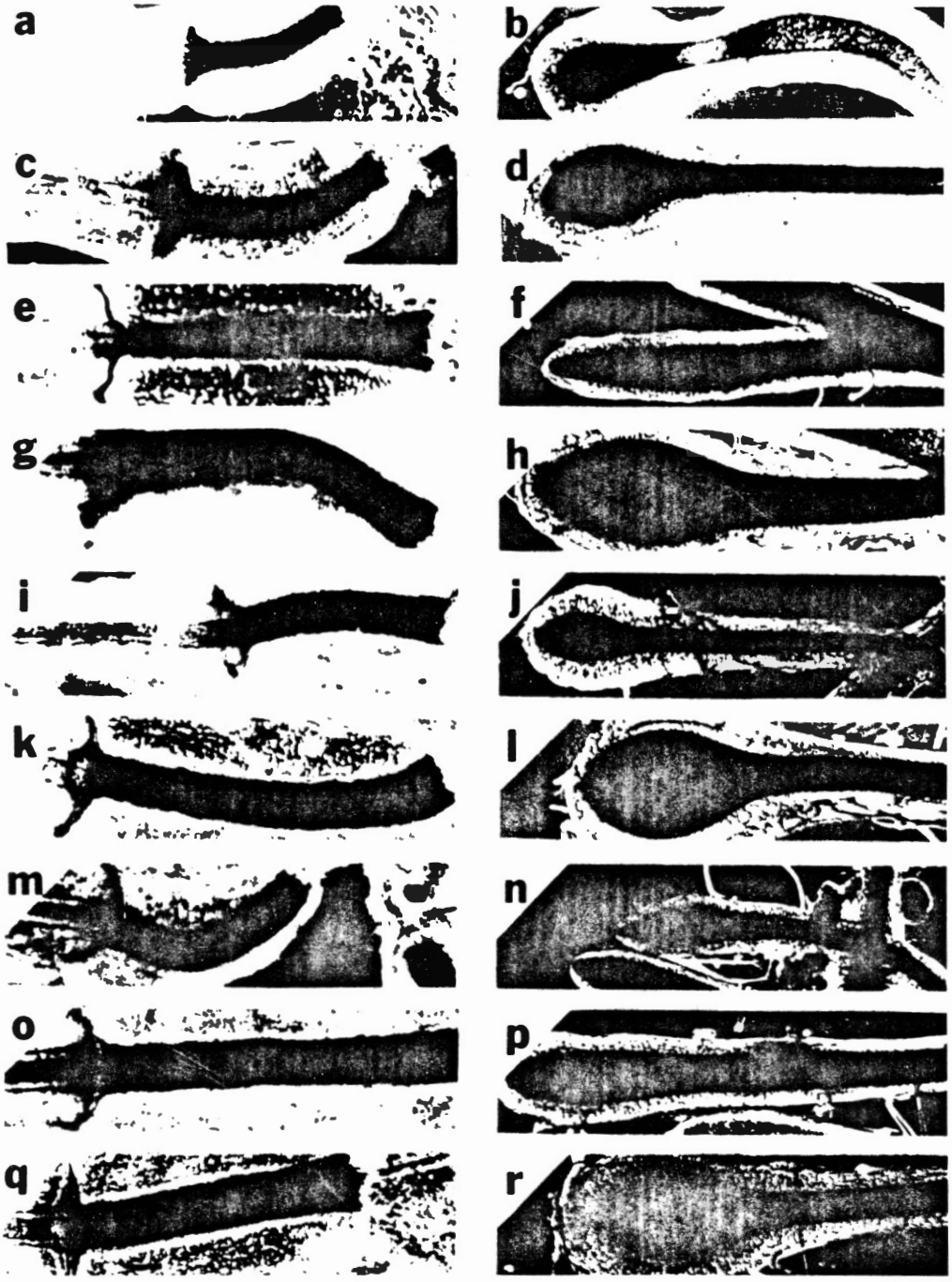
Figure 21n. Terminal Capsule of T. mularis;

Figure 21o. Sleeve of T. sulcifrons;

Figure 21p. Terminal Capsule of T. sulcifrons;

Figure 21q. Sleeve of T. calens;

Figure 21r. Terminal Capsule of T. calens.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Terminal capsule: diamond or occasionally ovate; smooth, intima heavily chitinous (Fig. 21h).

Tabanus abactor Philip

Sleeve: three prongs proximally; semi-circular cupule (Fig. 21i).

Duct: straight; not bulbous.

Terminal capsule: diamond or occasionally lanceolate; smooth, moderately chitinous (Fig. 21j).

Tabanus stygius Say

Sleeve: three prongs proximally; semi-circular cupule (Fig. 21k).

Duct: straight; not bulbous.

Terminal capsule: ovate or occasionally diamond; smooth, moderately chitinous (Fig. 221).

Tabanus mularis Stone

Sleeve: three prongs proximally; semi-circular cupule (Fig. 21m).

Duct: straight; not bulbous.

Terminal capsule: diamond; smooth, intima moderately chitinous (Fig. 21n).

Tabanus sulcifrons Macquart

Sleeve: three prongs proximally; semi-circular cupule (Fig. 21o).

Duct: straight; not bulbous.

Terminal capsule: diamond or occasionally lanceolate; smooth, intima heavily chitinous (Fig. 21p).

Tabanus calens L.

Sleeve: three prongs proximally; bulbous cupule with lateral

horn projections (Fig. 21q).

Duct: straight; not bulbous.

Terminal capsule: lanceolate or occasionally tubular; smooth, intima lightly chitinous (Fig. 21r).

Tabanus trimaculatus Palisot de Beavois

Sleeve: two prongs proximally; semi-circular or circular cupule (Fig. 22a).

Duct: straight; not bulbous.

Terminal capsule: lanceolate or occasionally diamond; smooth, intima moderately chitinous (Fig. 22b).

Tabanus quaesitus Stone

Sleeve: three prongs proximally; semi-circular cupule (Fig. 22c)

Duct: straight; not bulbous.

Terminal capsule: lanceolate or occasionally diamond; smooth, intima moderately chitinous (Fig. 22d).

Tabanus equalis Hine

Sleeve: three prongs proximally; semi-circular cupule (Fig. 22e).

Duct: straight, not bulbous.

Terminal capsule: tubular; smooth, intima moderately chitinous (Fig. 22f).

Chrysops flavidus Wiedemann

Sleeve: tapered point proximally; diamond cupule (Fig. 22g).

Duct: curved; bulbous.

Terminal capsule: lanceolate; intima with longitudinal impressions, moderately chitinous (Fig. 22h).

Figure 22. Photomicrographs of the Sleeve (200x) and Terminal Capsule (100x) Regions of Nine Species of Tabanidae.

Figure 22a. Sleeve of *Tabanus trimaculatus*;

Figure 22b. Terminal Capsule of *T. trimaculatus*;

Figure 22c. Sleeve of *T. quaesitus*;

Figure 22d. Terminal Capsule of *T. quaesitus*;

Figure 22e. Sleeve of *T. equalis*;

Figure 22f. Terminal Capsule of *T. equalis*;

Figure 22g. Sleeve of *Chrysops flavidus*;

Figure 22h. Terminal Capsule of *C. flavidus*;

Figure 22i. Sleeve of *C. callidus*;

Figure 22j. Terminal Capsule of *C. callidus*;

Figure 22k. Sleeve of *Silvius quadrivittatus*;

Figure 22l. Terminal Capsule of *S. quadrivittatus*;

Figure 22m. Sleeve of *Esenbeckia incisuralis*;

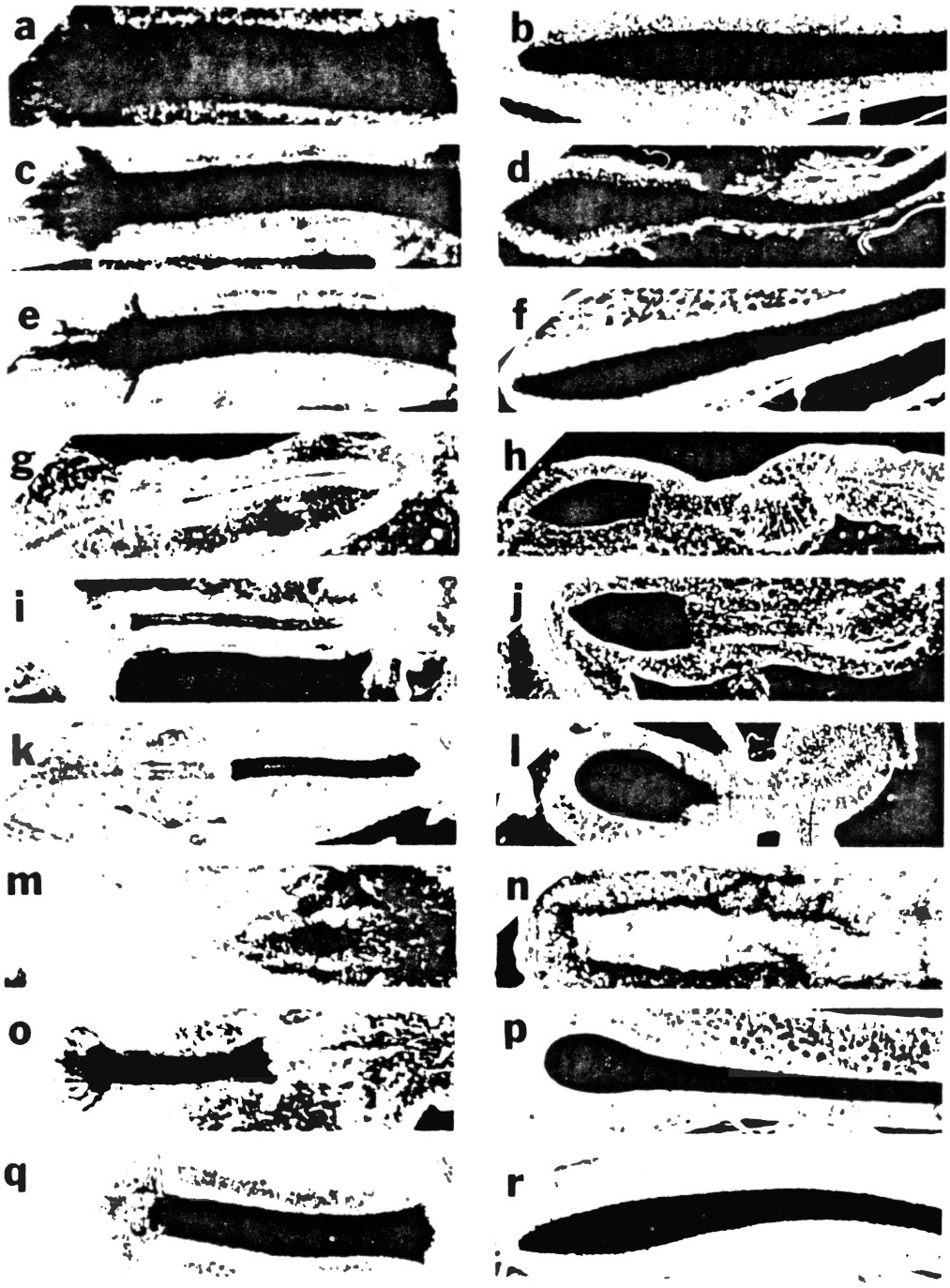
Figure 22n. Terminal Capsule of *E. incisuralis*;

Figure 22o. Sleeve of *Anacimas dodgei*;

Figure 22p. Terminal Capsule of *A. dodgei*;

Figure 22q. Sleeve of *Hybomitra lasiophthalma*;

Figure 22r. Terminal Capsule of *H. lasiophthalma*.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Chrysops callidus Osten Sacken

Sleeve: tapered point proximally; diamond cupule (Fig. 22i).

Duct: curved; bulbous.

Terminal capsule: lanceolate; sculptured, intima moderately chitinous (Fig. 22j).

Silvius quadrivittatus Say

Sleeve: tapered point proximally; flattened cupule (Fig. 22k).

Duct: straight; slightly bulbous.

Terminal capsule: ovate; smooth, intima highly chitinous (Fig. 22l).

Esenbeckia incisuralis Say

Sleeve: no distinct sleeve region (Fig. 22m).

Duct: straight, not bulbous.

Terminal capsule: tubular; smooth, non-chitinous intima (Fig. 22n).

Anacimas dodgei Whitney

Sleeve: three prongs proximally; spherical or kidney cupule (Fig. 22o).

Duct: straight; not bulbous.

Terminal capsule: ovate; smooth, intima moderately chitinous (Fig. 22p).

Hybomitra lasiophthalma Macquart

Sleeve: three prongs proximally; semi-circular cupule (Fig. 22q).

Duct: straight; not bulbous.

Terminal capsule: tubular or occasionally lanceolate; smooth, intima moderately chitinous (Fig. 22r).

The shape, size and certain morphological characteristics of the

spermatheca can be used to differentiate Oklahoma Tabanidae to species. A key for 18 species of Oklahoma Tabanidae was prepared based on the morphological descriptions of the spermathecae and the measurements of the spermathecae under magnification described in the Materials and Methods and listed in Tables I and II.

1. Terminal capsule without a chitinous intima; no distinct sleeve region present (Fig. 22 m,n). . . . . Esenbeckia incisuralis

   Terminal capsule with a chitinous intima; distinct sleeve region present . . . . . 2

2. Spermathecae with a diamond cupule; sleeve tapered to point proximally. . . . . 3

   Spermathecae with cupule other than diamond; sleeve does not taper to a point . . . . . 4

3. Terminal capsule intima with longitudinal impressions (Fig. 22h) . . . . . Chrysops flavidus

   Terminal capsule with sculptured intima (Fig. 22j) . . . . . Chrysops callidus

4. Spermathecae with flattened cupule (Fig. 22k) .Silvius quadrivittatus

   Spermathecae with non-flattened cupule . . . . . 5

5. Spermathecae with kidney cupule (Fig. 22p). . . . . Anacimas dodgei

   Spermathecae with cupule other than kidney . . . . . 6

6. Spermathecal duct curved . . . . . 7

   Spermathecal duct straight . . . . . 8

7. Spermathecal duct bulbous; spermatheca 1-1½ mm length (Fig. 21b) . . . . . Tabanus subsimilis

Spermathecal duct not bulbous; spermatheca 4-4½ mm length (Fig. 21g) . . . . . Tabanus atratus

8. Sleeve with two prongs proximally . . . . . .9

Sleeve with three prongs proximally . . . . . 10

9. Terminal capsule intima moderately chitinous; spermatheca 4-4½mm length (Fig. 21f) . . . . . Tabanus nigripes

Terminal capsule intima lightly chitinous; spermatheca 8-8½mm length (Fig. 22b) . . . . . Tabanus trimaculatus

10. Spermathecae with bulbous cupule with lateral horn projections (Fig. 21q) . . . . . Tabanus calens

Spermathecae cupule other than bulbous without lateral horn projections . . . . . 11

11. Spermathecae with inverted semi-circular cupule. . . . . 12

Spermathecae with semi-circular cupule . . . . . 13

12. Spermatheca length 3-3/4-4mm . . . . . Tabanus venustus

Spermatheca length 5-5½mm. . . . . Tabanus stygius

13. Terminal capsule tubular (Fig. 22e). . . . . Tabanus equalis

Terminal capsule other than tubular . . . . . 14

14. Terminal capsule only diamond (Fig. 21n) . . . . . .Tabanus mularis

Terminal capsule can be other than diamond . . . . . 15

15. Terminal capsule lanceolate or occasionally diamond (Fig. 22c) . . . . . Tabanus quaesitus

Terminal capsule diamond or occasionally lanceolate . . . . . .16

16. Terminal capsule intima moderately chitinous; 4-4-3/4mm length (Fig. 21j) . . . . . Tabanus abactor

Terminal capsule intima heavily chitinous; 6-1/4-6-3/4mm length (Fig. 21p) . . . . . Tabanus sulcifrons

This key shows that spermathecal characteristics of Oklahoma are sufficiently different to seperate flies to species. This offers an alternative way to identify tabanids which become either badly damaged, or difficult to seperate by external characteristics. Finally observations on spermathecae can be used as an aid in confirming identifications made by taxonomic characteristics which often are difficult to see, or have been damaged or destroyed.

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